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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/919,501	08/28/97	O'GORMAN S	SALK2190 (08

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EXAMINER	
WILSON, M	
ART UNIT	**PAPER NUMBER**
1633	25

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/919,501	Applicant(s) O'GORMAN ET AL.	
	Examiner Michael Wilson	Art Unit 1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 April 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *12-15,18-24,26,28-32,34-44 and 46-51* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12-15,18-24,26,28-32,34-44 and 46-51* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 remain under consideration in the instant application. Applicants' arguments filed 4-24-01, paper number 24, have been fully considered but they are not persuasive. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112

1. Claims 12-15, 18-24, 26, 28-32, 34-44 remain rejected and 46-51 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mouse ES cell whose genome comprises a construct comprising a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter and a method of making a transgenic mouse comprising implanting the mouse ES cells above into a host female such that a transgenic mouse is obtained, wherein said transgenic mouse expresses recombinase in its spermatid to a level that results in recombination in an embryo, does not reasonably provide enablement for any mammalian ES cell, any germline-specific promoter or method of making any transgenic animal or recombinant allele as broadly claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The specification is directed toward ES cells comprising a nucleic acid construct encoding a recombinase gene operatively linked to a germline-specific promoter to conditionally express a

gene of interest. The purpose of making such ES cells is to make transgenic mice expressing recombinase in their gametes such that the "marker gene would be excised in at least some of the progeny of ES cell chimeras" (page 3, line 3) or to "deliver recombined target nucleic acid constructs to the early embryo" (page 3, line 13).

The state of the art at the time of filing was such that the phenotype of transgenic animals was unpredictable due to the unpredictability of transgene expression (Mullins of record, 1996, J. Clin. Invest., Vol. 98, pages S37-S40; see page S37, column 2, line 7). The transgene may even be expressed but not functional (i.e. "silenced") (see page S37, column 2, line 7). Wall of record teaches transgene behavior was unpredictable because transgene expression often occurs in unintended tissues or at developmentally incorrect times (Wall, 1997, J. Dairy Science, Vol. 80, pages 2213-2224; see page 2216, column 1, "Transgene expression"). The instant claims relate to expressing recombinase in ES cells; however, if germline-specific recombinase activity were not sufficiently high to mediate recombination, embryos expressing recombinase would be mosaic and not display a mutant phenotype (Lewandoski of record, 1997, Current Biology, Vol. 7, pages 148-151; see page 151, column 1, line 4). Thus, recombinase expression may not alter the phenotype of the animal.

The specification discloses making ProCre transgenic mice by transfecting mouse ES cells with a nucleic acid construct comprising a sequence encoding Cre recombinase operatively linked to the mouse protamine 1 (MP1) promoter (page 19, line 25; page 21, line 3). ProCre transgenic mice were bred with transgenic mice containing a nucleic acid construct comprising a loxP-

flanked neomycin resistance gene and the β-gal gene disrupting the RNA polymerase II locus (P2Bc, Figure 1, see also page 22, line 10). ProCre/P2Bc male mice were bred with wild-type female mice resulting a majority of embryos carrying a recombined P2Bc gene, namely P2Br, which does not have the neomycin resistance gene (page 22, lines 22-30). Note that the specification refers to the results of the experiment in Table 1 on page 22, line 30; however, Table 1 is not present in the instant application. The specification does not teach making ES cells with ProCre and P2Bc or isolating ES cells from ProCre/P2Bc transgenic mice. ProCre males bred with P2Bc females did not result in recombination (page 23, line 17).

Heterozygous ProCre/P2Bc male mice demonstrated expression of P2Br in testes but not in kidney, brain or spleen when tested by Southern Blot analysis (page 24, line 4). However, testes tissue and only one other tissue selected from the group of kidney, brain or spleen was tested in each mouse (page 24, line 1). Upon further investigation, the heterozygous ProCre/P2Bc male mice expressed P2Br in the heart, brain and spleen as determined by a more sensitive PCR method (page 24, line 32; page 25, lines 8-13). Thus, heterozygous ProCre/P2Bc male mice expressed P2Br in testes, heart, brain and spleen.

The specification does not enable any non-human mammalian ES cells, any germline-specific promoter or a method of making any transgenic animal as broadly claimed. The specification does not teach making any ES cells other than mouse ES cells, any germline-specific promoters other than those in mice (page 6, lines 1-12) or any germline-specific promoter that

functions in a transgenic mouse to produce germline-specific recombinase expression a mouse other than the MP1 promoter.

Applicants argue that the promoters contemplated on page 6, lines 1-12 would function in the instant invention. Applicants argument is not persuasive because the specification has not provided adequate guidance indicating the promoters have equivalent function as MP1 in transgenic mice.

Applicants argue that MP1 is germline specific because it is equivalent to the substantially exclusive expression of the protein in the germline with a background expression in other tissues that is "substantially lower" (e.g. 100 fold lower) than germline tissues. Applicants argument is not persuasive because "germline specific" promoters as defined on page 8, lines 15-18 encompasses exclusive expression in the germline and no expression in other tissues, because the specification does not define "germline specific" promoters as those that direct expression to the germline and only allow 100 fold lower expression in other tissues and because the term "substantially" is not defined in the specification. Furthermore, the specification does not teach that the GH, NSE, GFAP, neurotransmitter, etc. on page 8, lines 15-18 direct expression to the germline.

Applicants discussion of the breadth of recombinase is noted; however, the scope of recombinases is not an issue of record.

Applicants argue that any number of constructs could be introduced into the ES cell. While any number of DNA constructs could be introduced into ES cells, applicants have not

provided adequate guidance for one of skill to use any such ES cells for reasons of record. The rejection is based on how to determine the phenotype of animals created from such ES cells.

Applicants argue that the phenotype of the animal obtained could be monitored by analyzing DNA expression. Applicants argument is not persuasive because the level of expression may not be adequate to alter the phenotype of the animal and because the resulting phenotype could not be predicted. Applicants argue that it is not necessary to predict the level of recombinase expression or the phenotype of the animal because they are determined after introducing the DNA. Applicants argument is not persuasive because the purpose of the specification is to guide the artisan how to make and use the invention. Applicants do not overcome the unpredictability in the art by teaching the phenotype of the resulting transgenic animals. Applicants argue that the ES cells are useful in making transgenic animals with an array of mutations.

Claims reciting the limitation of an ES cell comprising a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter and further comprising 1) a nucleic acid fragment flanked by two recombinase target sites, 2) a nucleic acid construct encoding recombinase operatively linked to an inducible promoter or 3) a nucleic acid construct encoding recombinase operatively linked to a tissue-specific promoter are not enabled (claims 13-15, 26, 28-32, 34-43, 46-48). The specification does not teach transfecting any ES cells with two constructs of any kind such that the phenotype of the resulting animal could be determined. The specification does teach isolating male ES cell lines from the ProCre mice and transfecting them

with a vector comprising a selectable marker flanked by two loxP sites (page 25, lines 22-34; page 26, lines 1-7). A portion of the transfected ES cells recombined such that the selectable marker was removed in some of the ES cells (page 26, lines 7-26). However, the specification does not teach how to use such ES cells or the phenotype of the resulting transgenic mouse is different than the wild-type. The specification does not provide a use for ES cells or mice expressing a marker gene. The specification also contemplates using an inducible promoter to facilitate temporal control of recombinase expression in ES cells (page 19, line 10). The specification does not teach the phenotype of any transgenic animal obtained using an inducible promoter. Given the unpredictability in the art regarding the phenotype of transgenic animals, the specification does not enable ES cells comprising a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter and further comprising 1) a nucleic acid fragment flanked by two recombinase target sites, 2) a nucleic acid construct encoding recombinase operatively linked to an inducible promoter or 3) a nucleic acid construct encoding recombinase operatively linked to a tissue-specific promoter as claimed.

Applicants argue that the specification enables an array of mutations on page 15, lines 16-35. Applicants argument is not persuasive because applicants do not provide the resulting phenotype obtained in animals made with such mutations.

The methods of claims 28-32, 34-44 and 46-51 are not enabled because the excision of a selectable marker in an ES cell, the production of recombinant alleles, the conditional assembly of

genes or generating recombinant livestock as claimed do not result in altering the phenotype of the ES cell or transgenic animal for reasons of record.

Claim 43 is not enabled because the specification does not teach any inactive gene segments which can be used to make a eukaryotic cell of interest by merely introducing the segmenting to an ES cell as claimed. It cannot be determined from the specification what steps and DNA segments are required to obtain a biologically active expression product upon passage of the genome through gametogenesis and conditional assembly of functional genes as claimed.

Applicants argue that "inactive gene segments" in claim 43 are enabled on page 15, lines 30 through page 16, line 4. Applicants argument is not persuasive because page 15, lines 30 through page 16, line 4 does not teach how to use inactive gene segments.

2. Claims 12, 28-32, 34-44 and 46-51 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 28-32 and 34-39 are indefinite because the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is unclear. Applicants argue that the phrase is consistent with use in the field and the specification defines the phrase as spermatogenesis or oogenesis (page 11, lines 30-32). Applicants argement is not persuasive. It is unclear how "passaging the genome derived from said embryonic stem cells through gametogenesis" relates to "spermatogenesis" or "oogenesis" as on page 11, lines 30-32. The specification does not describe how a genome is "derived" from an ES cell or how the genome is

"passaged" through gametogenesis. It is unclear if applicants intend to claim obtaining an animal from the ES cells and breeding the animal such that the transgene is present in the gametes or whether the transgene is present until gametogenesis, spermatogenesis or oogenesis occurs.

Claims 12, 26, 32 and 34-39, 43 are indefinite because the "introducing" step in claims 32, 34, is unclear. Similarly, claims 35-39 are indefinite because the phrase "introducing a nucleic acid fragment... ... embryonic stem cells of claim 26" is unclear. It is unclear if the nucleic acid fragment introduced in claims 32 and 34-39 is the nucleic acid construct in the ES cells referred to in the parent claims or if it refers to a second construct that is introduced into the cell.

Claim 43 remains indefinite because the body of the claim does not reflect the preamble of the claim by requiring conditional assembly of functional gene(s). It is unclear whether the "DNA" produced as newly amended correlates with the inactive gene segments or some other DNA present in the cell.

Claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 appear to be free of the prior art of record because the prior art of record did not teach or suggest a mammalian ES cell transfected with a construct comprising a nucleic acid sequence encoding recombinase operatively linked to a germline-specific promoter, wherein the construct is in the genome of the cell and the recombinase is not expressed in the stem cell, a mammalian ES cell comprising a construct comprising a nucleic acid sequence encoding recombinase operatively linked to a germline-

specific promoter and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells or methods of using such ES cells.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

No claim is allowed.

Inquiry concerning this communication or earlier communications from the examiner should be directed to Michael C. Wilson who can normally be reached on Monday through Friday from 9:00 am to 5:30 pm at (703) 305-0120.

Questions of formal matters can be directed to the patent analyst, Tracey Johnson, who can normally be reached on Monday through Friday from 9:00 am to 5:30 pm at (703) 305-2982.

Questions of a general nature relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-1235.

If attempts to reach the examiner, patent analyst or Group receptionist are unsuccessful, the examiner's supervisor, Deborah Clark, can be reached on (703) 305-4051.

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